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STUDIES ON LIVER DNA IN TRITIATED KANGAROO RATS LIVING AT SEDAN CRATER

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Contents

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Abstract

Kangaroo rats (Dipodomys merriami) living near the crater of an old nuclear detonation were found to be uniformly tritiated. The lifetime chronic dose to adult animals was estimated to be 10 rad from internal ^{3}H and 10 rad from persistent external β- and γ-emitting radionuclides.

Liver DNA was isolated from these animals and from control animals trapped in areas not enriched in radionuclides. DNA from the crater animals contained 6×10^{6} dpm/g hydrogen.

In comparison with control DNA, the crater DNA exhibited the following: more protein and histone, higher molar absorbance at 260 mμ, lower thermal transition magnitude with a small secondary transition, lower mole fraction of A + T nucleotides, increased template activity for 2 RNA polymerases and 1 DNA polymerase, and relative increase in ATP-γ-^{32}P incorporation.

There was no difference in the molar absorbance after heat denaturation, thermal transition midpoint, intrinsic viscosity before or after heating, or susceptibility to hydrolysis by DNase I.

The physicochemical observations are compatible with a slight degree of unfolding or disorganization in the structure of the DNA of the crater animals. The enhanced template activity may have resulted from the introduction of additional binding sites for the polymerases by the radiation exposure or by subsequent repair mechanisms.

Thus a radiation effect at a very low chronic radiation exposure has been demonstrated with biochemical techniques. We cannot conclude that the changes were harmful, because the population in which they were observed was apparently a healthy one. However, we must conclude that the changes are potentially harmful because they involve DNA, which plays a central role in normal and abnormal cell behavior. The exposure to radiation in this study approached levels which could be encountered in peaceful applications of nuclear energy.

Introduction

Deoxyribose nucleic acid (DNA)* situated in the nucleus of cells performs two major functions which place it in the key position to control both the present and the future behavior and well-being of the cell. DNA serves as a primer and template firstly for self-duplication during the process of cell division and secondly for the synthesis of several forms of ribose nucleic acid (RNA) which are involved in the day-to-day control of metabolism in the cell (Fig. 1). It is generally believed that DNA is the cellular constituent that is most sensitive to damage by ionizing radiations. Such damage is thought to be responsible for the phenomena most readily observed in mammalian organisms as consequences of radiation exposure, namely, chromosome defects,[1] delay in cell division,[2] and cell death.[3,4] The arguments favoring DNA as the prime target for radiation action have been discussed by Hutchinson[5] and by Haynes.[6]

With biochemical techniques we are seeking evidence for molecular changes that may be related to the morphologic and functional effects of ionizing radiations. A number of authors have published reports which suggest that the

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*The following abbreviations are used throughout this report:

DNA	deoxyribose nucleic acid;
RNA	ribose nucleic acid;
DNase I	deoxyribonuclease I (pancreatic);
PEI	polyethyleneimine;
A_{260}	absorbance at 260 mμ;
E(P)	absorbance at 260 mμ per mole of nucleotide phosphate per liter;
Tm	midpoint temperature of major thermal transition of ultraviolet absorbance;
Tm'	approximate midpoint temperature of secondary thermal transition;
$T_{1/2}$	width of thermal transition expressed as the temperature difference between 1/4 and 3/4 of the total transition of ultraviolet absorbance;
$[\eta]$	intrinsic viscosity;
ATP-^{14}C	adenosine-5'-triphosphate uniformly labeled with ^{14}C;
dCTP-^{14}C and dTTP-^{14}C	deoxyribonucleoside triphosphates labeled in the pyrimidine moiety;
ATP-γ-^{32}P	adenosine-5'-triphosphate labeled in the terminal phosphate only with ^{32}P;
A, T, G, C	deoxyribonucleotides of adenine, thymine, guanine and cytosine, respectively, when present in DNA or polynucleotide;
ϕ	mole fraction A + T/A + T + C + G in DNA;

abbreviations of other nucleic acid constituents conform to standard usage.

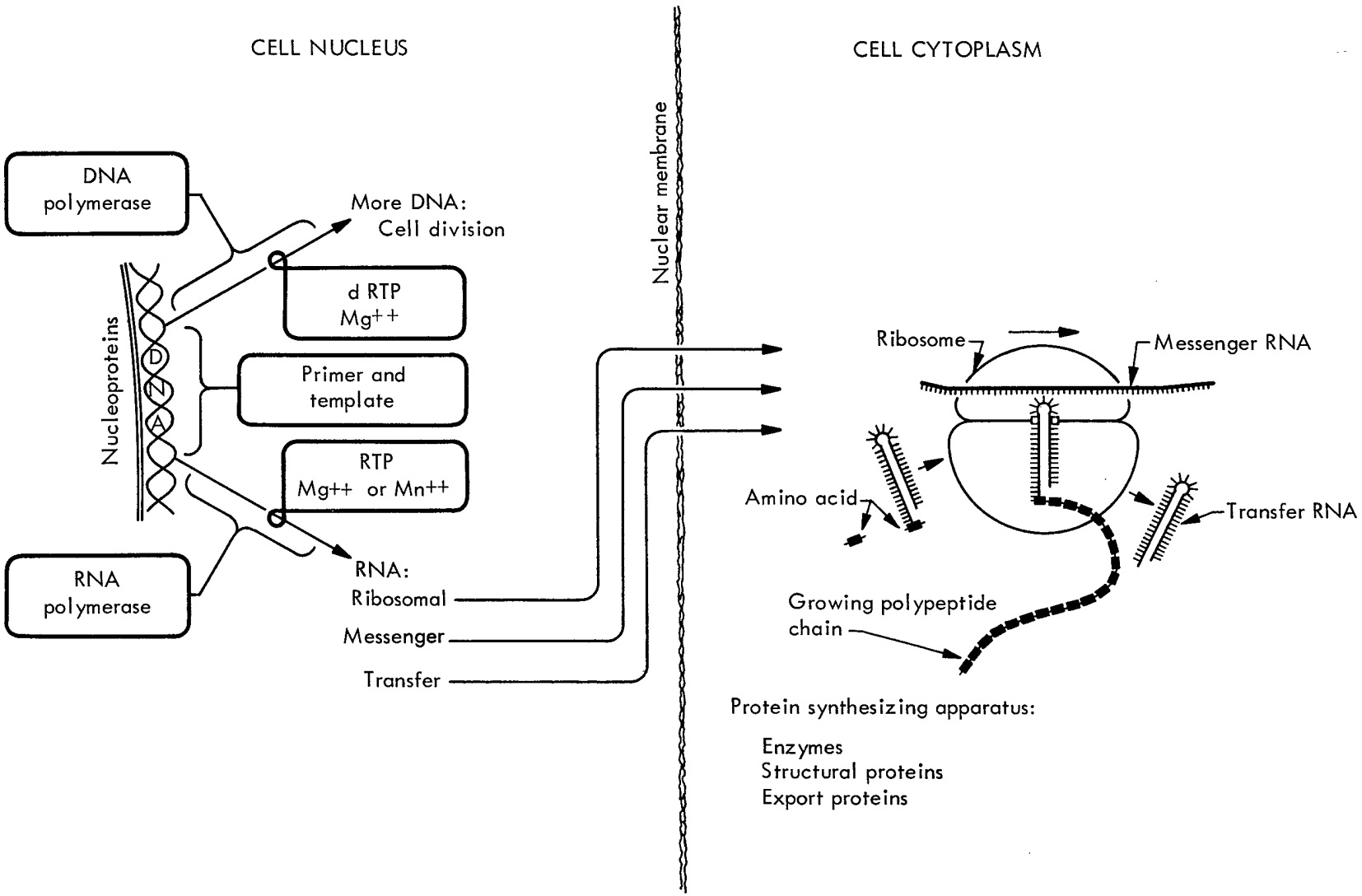

Fig. 1. Schematic representation of the dual role of DNA in the cell as primer-template for (1) DNA replication when the cell divides, and (2) RNA transcription for continuous regulation of protein synthesis and metabolism. The abbreviation dRTP refers to the four deoxyribonucleotide triphosphates; RTP refers to the four ribonucleotide triphophates. Adapted from "Antibiotics and the Genetic Code," Luigi Gorini, Sci. Am. 214 (4), 103 (1966).

primer-template functions of DNA are very sensitive to radiations (discussion below) and to chemical carcinogens[7] and may also be altered in animal and human tumors.[8-10]

An interesting experiment in the interactions of man and nature presented itself in the course of studies by this laboratory on the flora and fauna reentering the region around the crater of an old nuclear detonation.[11,12] As a result of the combined processes of nuclear fission and fusion the environment acquired an essentially uniform distribution of tritium together with a small amount of persistent radionuclides emitting hard β and γ radiations. The principal mammalian species present, the kangaroo rats,[13] had lived their entire lifetimes in this tritiated environment, so that all body consitituents were similarly labeled.[14] In addition these animals received a comparable radiation dosage from penetrating external radiations. The features of particular interest in this study were the chronic irradiation at a low dose level, and the especially low-energy β radiation from the tritium, which was distributed throughout the body water and tissues of the animals.

Liver DNA was purified from these irradiated animals and from control animals of the same species living in an uncontaminated environment. The DNA preparations were compared with respect to template activity for several polymerase enzymes and with respect to a number of physical and chemical properties

The results of this study demonstrated that DNA prepared from the irradiated animals exhibited a small but consistent increase in its primer-template function for several polymerases. The irradiated DNA exhibited an increased protein content after a standard purification procedure and slight but consistent alterations in the thermal transition and in the ultraviolet spectrum taken after heat denaturation. At present we are unable to conclude whether these changes represent a deleterious effect of radiation exposure. The observations are significant, however, because the changes occurred at a dose level considerably lower than that of most previously observed effects of ionizing radiation.

Materials and Methods

ANIMALS

Kangaroo rats (Dipodomys merriami) (Fig. 2) were trapped alive within a distance of 200 yards from the Sedan crater (Fig. 3) at the Nevada Test Site of the U. S. Atomic Energy Commission. This crater resulted from the detonation of a thermonuclear device in 1962, five years before the animals were obtained. The species of kangaroo rat was known to have reentered the vicinity of the crater approximately two years before sampling. Control animals of the same species were trapped near Camp Mercury approximately 40 miles south of the Sedan crater in an area not known to be contaminated by any radioactive materials.

The animals were caged and shipped by air to our laboratory. They were killed

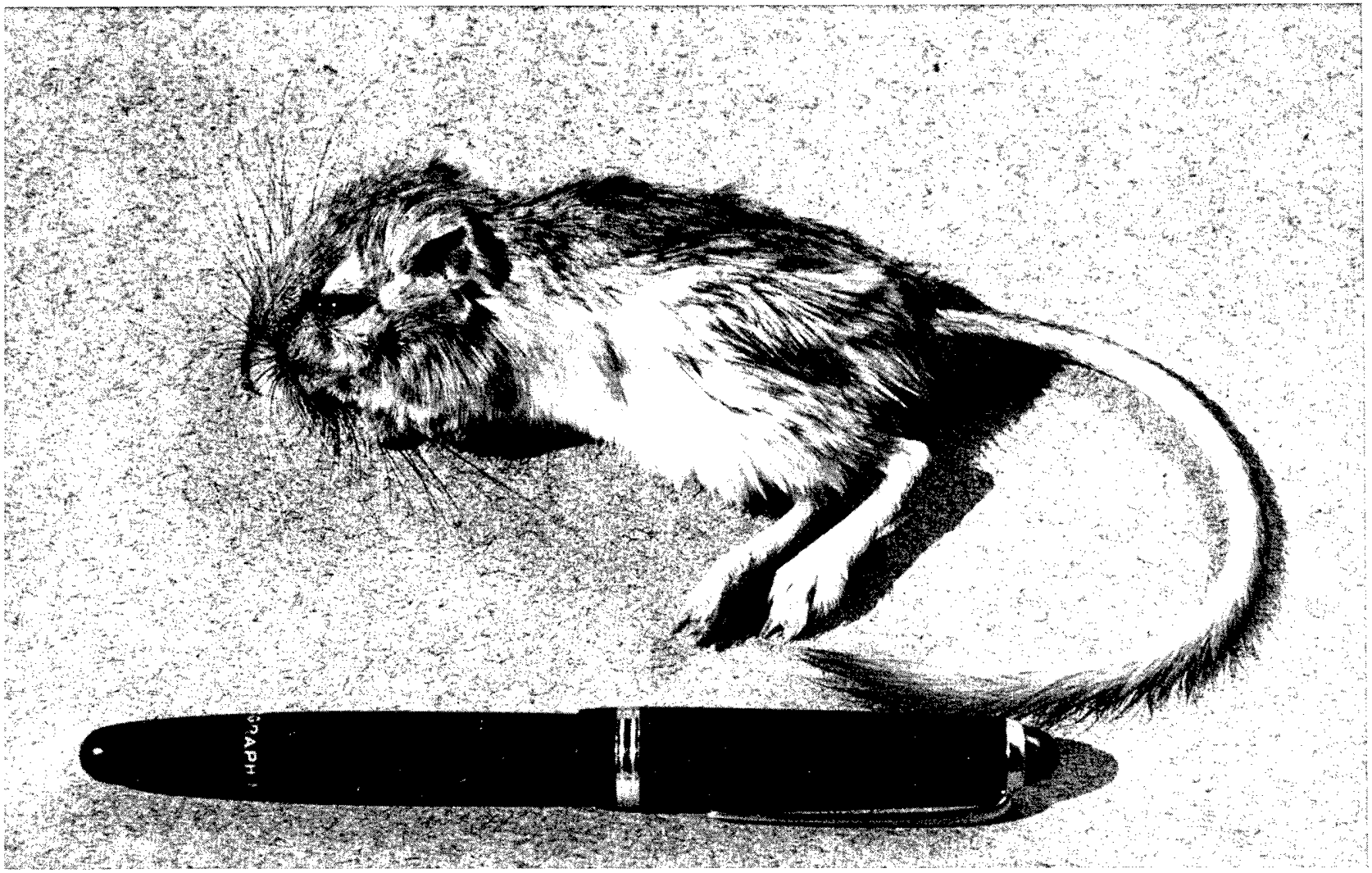

Fig. 2. Adult kangaroo rat (Dipodomys merriami). Average weight, 38 g.

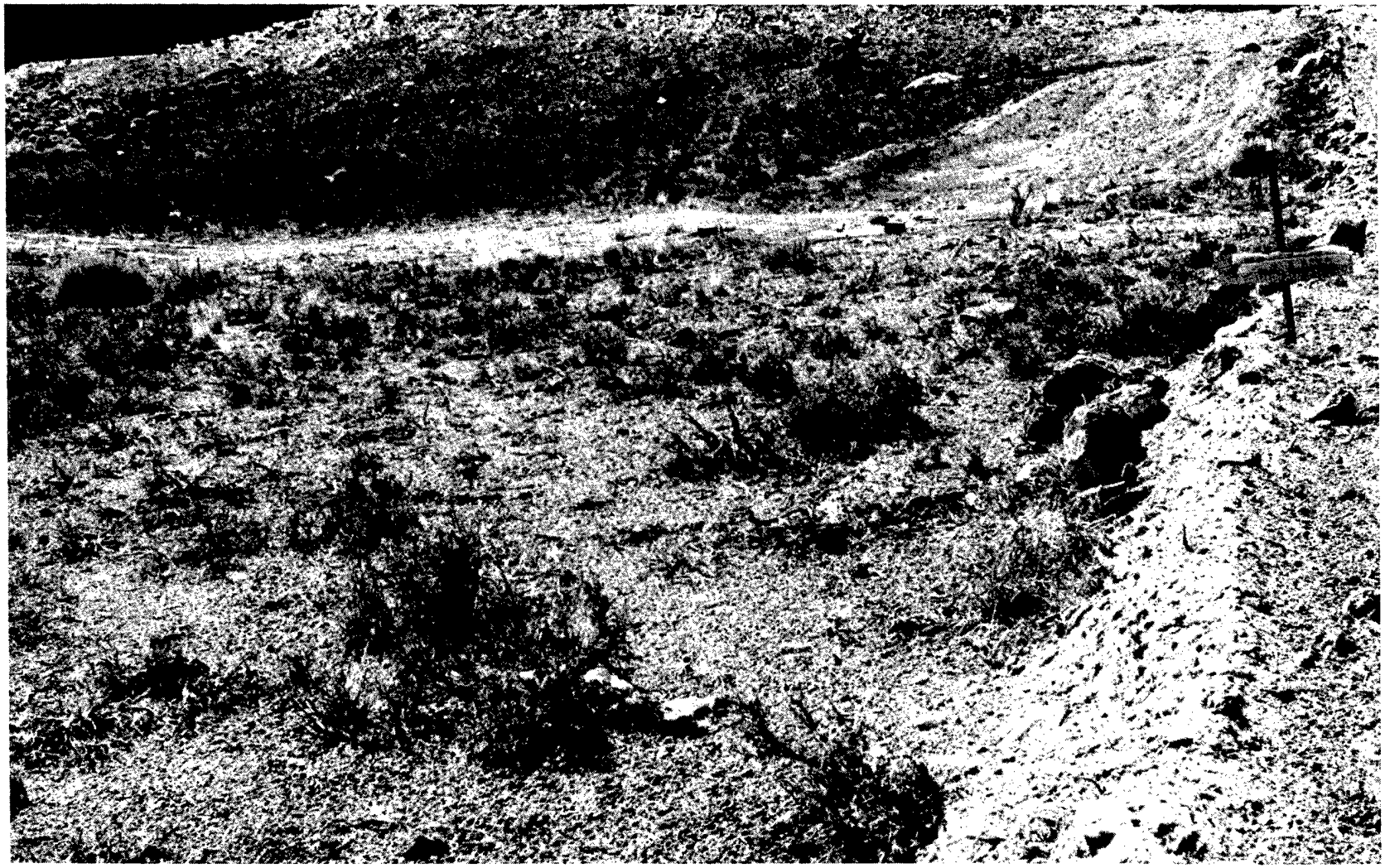

Fig. 3. Throwout zone surrounding Sedan crater, five years after detonation. The predominant vegetation is Russian thistle, Salsola kali, upon which the kangaroo rats live. Several box traps for live-trapping are visible in the background.

by ether or by cervical dislocation. The livers were quickly excised and placed on ice and those from four to five animals were pooled before the isolation of DNA.

TRITIUM CONTENT

The ^{3}H content of body water and the organic hydrogen of tissues were determined by methods developed by Koranda et al.[12]

DNA ISOLATION

The excised livers were pooled and passed through a tissue press (Harvard Apparatus Company, Millis, Massachusetts). The pressed material was frozen and thawed once. DNA was isolated by the method of Kay, Simmons and Dounce.[15] The published procedure was modified only in that after the initial homogenization steps, the solution volumes were decreased in accordance with the smaller yield of DNA from liver than from calf thymus. The final yield of dried DNA was approximately 1 mg per gram of liver. The air-dried DNA was stored at -20°C.

Solutions of DNA were made in 0.015 M sodium chloride/0.0015 M sodium citrate, 1/10th the "standard" concentration.[16] Concentrations of DNA were routinely determined by absorbance at 260 mμ. Stock solutions contained approximately 1 mg/ml and were stored at 4°C.

The kangaroo rat DNA was compared with Long-Evans rat liver DNA isolated by the same procedure and with salmon sperm DNA purchased from Calbiochem, Los Angeles, California.

ENZYMES

RNA polymerase and DNA polymerase prepared from E. coli were purchased from Biopolymers, Inc., Pinebrook, New Jersey. RNA polymerase from Micrococcus lysodeikticus was purchased from Miles Laboratories, Inc., Elkhart, Indiana. DNA polymerase was prepared through the pH 5 precipitation step from regenerating Long-Evans rat livers by the procedure of Mantsavinos.[17] Pancreatic DNase I (electrophoretically purified) and snake venom phosphodiesterase were purchased from Worthington Biochemical Corp., Freehold, New Jersey.

NUCLEOTIDES

Ribose and deoxyribose nucleotide triphosphates for the assays of primer-template function of the polymerases were purchased from P. L. Biochemicals, Inc., Milwaukee, Wisconsin. ^{14}C- and ^{3}H-labeled nucleotides were purchased from New England Nuclear Corp., Boston, Massachusetts. ATP-γ-^{32}P was purchased from International Chemical and Nuclear Corp., City of Industry, California.

MEASUREMENTS ON DNA

1. Protein Content

The protein content of DNA samples was determined by the method of Lowry et al.[18] with bovine serum albumin and a commerical histone preparation as standards.

2. Histone

The histone content of DNA samples was determined by the method of Sakaguchi

as described by Hamilton.[19] Standard solutions were made from arginine and from a lysine-rich histone fraction purchased from Calbiochem.

3. Nucleotide Composition

DNA samples were dissolved in 0.03 M $MgSO_4$, denatured for 10 min at 100°, and hydrolyzed in two stages by a modification of the method of Cohn et al.[20] Treatment with DNase I [DNA:enzyme 50:1 (w/w)] was carried out at pH 5-6 (unadjusted) and 37°C for 2 hr. The pH was then adjusted to 9.0 with NaOH and phosphodiesterase (DNA:enzyme 50:1 w/w) was added. Incubation at 37°C was carried out for 4 hr; after 1 hr the pH was readjusted with NaOH to 8.5-9. The total increment of A_{260} during hydrolysis was 70-75% of the native DNA value. The hydrolysates were concentrated under a stream of N_2. Aliquots were analyzed by thin-layer chromatography on PEI-cellulose by the method of Randerath.[21]

4. E (P)

Phosphorus was determined by the ultramicromethod of Bartlett.[22] The absorbance of the DNA solution at 260 mμ was determined and the extinction per mole of phosphorus was calculated.

5. Ultraviolet Absorption Spectrum

Spectra were determined in a Cary Model 15 recording spectrophotometer (Applied Physics Corp., Monrovia, California) and the wavelength of maximum absorption was estimated from the tracing to the nearest 1/2 mμ. The wavelength calibration of the instrument was checked with a solution of adenosine.

6. Thermal Transition

Thermal transition curves were determined in a Gilford Model 2000 recording spectrophotometer (Gilford Instrument Labs., Inc., Oberlin, Ohio) equipped with a Haake circulating bath (Brinkmann Instruments, Inc., Westbury, New York) and a Heller motor-controller (G. K. Heller Co., Las Vegas, Nevada) for programming a rise or fall of temperature. Temperature was recorded from a thermistor located in the sample compartment. The rate of temperature change was approximately 0.4°C/min. The midpoint of the thermal transition, T_M, and the temperature range between one-quarter and three-quarters of the total transition in absorbance at 260 mμ, $T_{1/2}$, were determined from the recording.

7. Spectral Analysis

A spectral analysis was performed by the method of Hirschman and Felsenfeld[23] at the beginning and at the end of the thermal transition procedure. The data were transferred to punched cards and the calculations were carried out with a CDC-6600 computer. The "true" concentration and mole fraction of A + T/A + T + G + C of the DNA solutions were calculated from the denatured spectrum. The molar extinction coefficients at 260 mμ at 26°C and above the thermal transitions were calculated from the A_{260} values at the two temperatures and the calculated concentrations. Absorbance ratios at 230 mμ/260 mμ and 290 mμ/260 mμ were calculated because these values appeared to be affected by radiation in these and other experiments.

8. Viscosity

Viscosity of DNA solutions was measured with a Zimm-Crothers low-shear viscometer (Beckman Instruments, Inc., Spinco Division, Palo Alto, California).[24,25] The viscosity relative to dilute saline-citrate buffer was measured at several concentrations of DNA between 10 and 100 μg/ml. The reduced specific viscosities and the intrinsic viscosity were calculated from programs developed for the Programma 101 desk computer (Olivetti-Underwood Corp., New York, New York). The slope of the least squares regression line for reduced specific viscosity vs concentration was also computed.

9. Tritium Content

Small samples of DNA were weighed on a Cahn microbalance (Cahn Instrument Co., Paramount, California) and were ashed in a Schöniger combustion flask. The $^{3}H^{2}HO$ produced was measured by liquid scintillation counting in Bray's solution.[26]

10. Reaction with Ethidium Bromide

The intensification of fluorescence quantum yield resulting from the interaction of DNA with ethidium bromide was determined in an Aminco-Bowman fluorometer (American Instrument Co., Silver Spring, Maryland) by the method of LePecq and Paoletti.[27,28]

ENZYME ASSAYS

The primer-template function of samples of kangaroo rat DNA was determined with several polymerase enzymes. The RNA polymerase from E. coli was assayed according to the method of Chamberlin and Berg.[29] The RNA polymerase from M. lysodeikticus was assayed by the method of Nakamoto et al.[30] ATP-^{14}C was used as the labeled precursor. The DNA polymerase from E. coli was assayed according to instructions given by Biopolymers, Inc., representing a modification of the method of Richardson et al.;[31] and the DNA polymerase from rat liver was assayed by the method of Mantsavinos.[17] For DNA polymerase assays dCTP-^{14}C or dTTP-^{14}C were used as labeled precursors.

The relationship between chain initiation and total RNA synthesis by RNA polymerase was determined by the method of Maitra et al.[32] The ATP-γ-^{32}P and the ATP-^{14}C were used in the same incubation vessel and a channel ratio method was used to determine the incorporation of each isotope into acid-insoluble polynucleotide.

The rate of hydrolysis of DNA by DNase I was followed measuring absorbance at 260 mμ in a Gilford recording spectrophotometer.[33]

Results

ESTIMATION OF RADIATION DOSAGE FROM ^{3}H

The measured ^{3}H content of the body water and the tissue organic material is given in Table I. In the crater animals this was found to be approximately 0.2 μCi/g. It is significant that the body water of the control animals was about

8 times the counting instrument background, although the soil water and plants of the area were below the detection limit in ^3H. Whether this is due to unrecognized contamination or selective uptake from the environment is under study, but in either case the radiation dosage from ^3H in the control animals would be extremely small.

The approximate tissue dose in the crater kangaroo rats was 2.9 mrad per hour or 25 rad per year (Table II). Since French et al.[34] have reported a mean lifetime of 4-5 months for these animals in their desert environment, the total radiation dose to adults was in the neighborhood of 10 rad. It was estimated that on the average there were 1.5 ^3H disintegrations per year in the DNA of each cell.

OTHER RADIATIONS

At the ground surface and at the depth of the burrows the persistent radionuclides around the crater delivered a γ

Table I. ^3H content of kangaroo rat body water, liver and DNA.

Kangaroo rat pool	Body water dis/min g H	Liver[a] dis/min g H	Liver DNA[a] dis/min g H
Crater A	5.6×10^6	6.0×10^6	6.2×10^6
Crater B	8.0×10^6	–	8.2×10^6
Control	5.5×10^3	–	$< 1.8 \times 10^5$[b]

[a]Tissue organic hydrogen measured by combustion and liquid scintillation counting after complete dehydration of the sample in vacuo.[12]

[b]The activity was below the estimated minimum detectable activity, because only 1 mg of DNA was available for combustion and liquid scintillation counting.

Table II. Calculation of ^3H irradiation dosage to kangaroo rat liver DNA.

Mean tissue ^3H activity	Approximately 5×10^5 dis/min/g tissue or 2.3×10^{-7} Ci/g.
Assumptions for dose estimation	1. ^3H is uniformly distributed in cells. 2. DNA absorbs energy uniformly with all other cell consitituents.
Estimated tissue dose from ^3H	Assumption: 1 Ci/g delivers 12×10^3 rad/hr. Tissue dose: 2.9 mrad/hr or 25 rad/yr.
Irradiation of DNA on a per-cell basis	Assumption: 6×10^{-12} g DNA/cell. Dose to DNA: (7 erg/g-day) $\times$ (6×10^{-12} g DNA/cell)/(1.6×10^{-12} erg/eV) = 26 eV or nearly 1 ion-pair/cell DNA/day.
^3H disintegration rate on a per-cell basis	4.2×10^{-3} ^3H disintegrations/cell DNA/day. 1.5 ^3H disintegrations/cell DNA/year.

dose of 2 mrad/hr and a hard β dose of 0.5 mrad/hr. Data from intermittent monitoring of the area showed little change in dose level throughout the sixth year after the detonation, during which the kangaroo rats were collected. The total external dose to the animals was therefore nearly equal to the dose from internal ^{3}H.

PROPERTIES OF LIVER DNA FROM CONTROL KANGAROO RATS: COMPARISON WITH DNA OF OTHER SPECIES

The properties of liver DNA from the kangaroo rat have not been reported previously. The protein and histone contents were similar to those found in liver DNA from the Long-Evans rat and in salmon sperm DNA (Table III). The absorbance at 260 mμ per mole of phosphorus content [E(P)] was equal to that of salmon sperm DNA (Table III). The value was slightly higher than that for liver DNA from the Long-Evans rat, but all of the E(P) values were within the range reported for native DNA samples from a variety of organisms. The ultraviolet absorption spectra of all the DNA samples examined were very similar, as were the thermal transition midpoints (Tm) in DSC buffer (Table IV). The intrinsic viscosities [η] of the DNA samples were equal within the limits of error of our technique, except for a higher value for heat-denatured DNA from the Long-Evans rat (Table VI).

The primer-template activity of kangaroo rat liver DNA was substantial when assayed in vitro with 4 different polymerases (Table VII). The values observed were similar to those of Long-Evans rat and salmon sperm DNA.

COMPARISON OF LIVER DNA FROM CRATER AND CONTROL ANIMALS

Several differences which may be interrelated have been shown in the properties of liver DNA from the tritiated and control animals.

Protein and Histone Content

Liver DNA from crater animals had approximately 6 times more protein and histone content than that from control animals (Table III).

Table III. Properties of DNA samples.

		Kangaroo rat		Long-Evans rat	Salmon sperm
		Crater	Control		
Absorbance 260 mμ, E(P)	$\left(\frac{A}{\text{mole P}}\right)$	6600	6920	6250	6920
Absorbance peak, λ_{max}	(mμ)	257.5	258	255	258
Protein content[a]	(wt %)	5.5	0.9	0.7	0.9
Histone content[a]	(wt %)	6.5	1.1	2.8	1.3

[a]The higher values for histone than for total protein content are considered to be the result of standardizing the protein method against bovine serum albumin and the histone method against a commercial sample of "lysine-rich histone." Within each method the comparison of different DNA samples should be valid, but the absolute values are not certain.

Nucleotide Composition

Preliminary experiments with thin-layer chromatography have not revealed a significant difference between the compositions of DNA from crater and control animals, nor evidence of altered nucleotides in detectable amounts in the tritiated samples. However, see the section below on analysis of ultraviolet absorption spectra.

Thermal Transition

The thermal transition midpoints (Tm) of crater and control DNA samples were approximately equal when the determinations were made in either of two buffers (Table IV). However, the percent increase in $A_{260\ m\mu}$ during the transition and the $T_{1/2}$, or width of the transition, were less in the crater samples (Fig. 4). The data in Fig. 4 are expressed in terms of molar absorbance calculated from the $A_{260\ m\mu}$ at each temperature and the concentration of DNA derived from the spectral data on denatured DNA.[23] Two significant features of the curves are brought out in this way. First, the crater DNA at low temperature exhibited a higher molar absorbance than the control sample, whereas the final absorbances of both samples after heating were similar. This phenomenon was observed repeatedly in comparing different samples of crater and control DNA. Second, the crater DNA samples exhibited a small second transition (Tm') about 10°C above the Tm in both buffer systems, which was either lacking or very small and indefinite in the control DNA. The Tm' was also not observed in the thermal transitions of Long-Evans rat or salmon sperm DNA.

Analysis of Ultraviolet Spectra

The method of Hirschman and Felsenfeld[23] was applied for analysis of the thermal transition of the ultraviolet absorption spectra of crater and control

Table IV. DNA thermal transition data.

DNA	Buffer	Tm (°C)	$\Delta\%$ $A_{260\ m\mu}$	T 1/2 (°C)	Tm' (°C)
Kangaroo rat					
Crater	DSC[a]	69.2	27.7	4.9	80.5
Control	DSC	69.5	33.1	5.2	none
Crater	G[b]	47.3	39.0	4.7	57.3
Control	G	47.2	47.1	5.8	63.8[c]
Long-Evans rat	DSC	68.6	38.5	4.3	none
Salmon sperm	DSC	69.2	35.1	4.2	none

[a]DSC buffer contains NaCl, 0.015 M, and Na citrate, 0.0015 M, pH 6.8 (the dilute saline-citrate of Marmur[16]).

[b]G contains NaCl, 0.001 M; Tris-Cl, 0.001 M; methanol, 51% (v/v); pH 7.1 (Geiduschek[35]).

[c]A small and indefinite second transition.

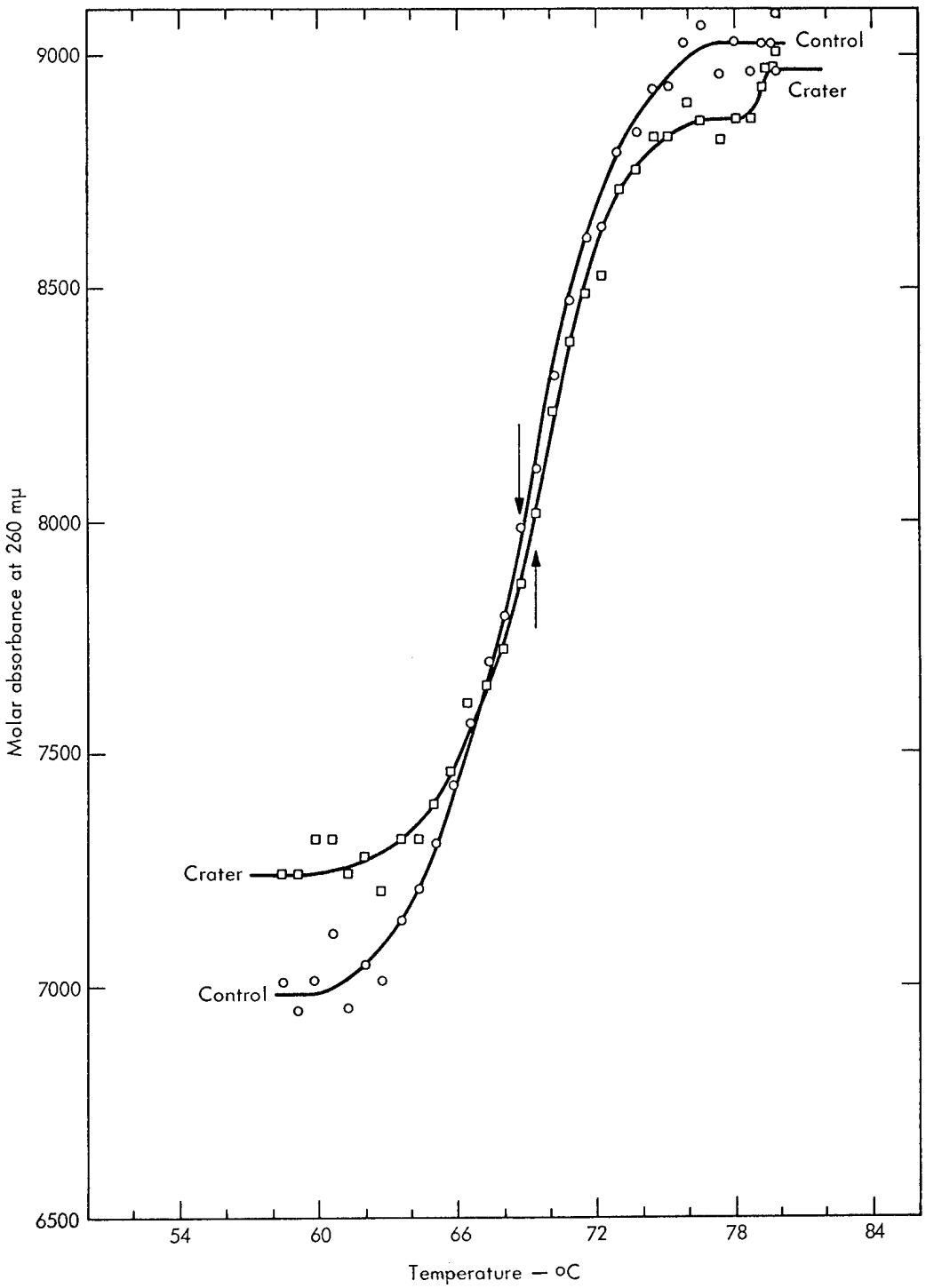

Fig. 4. Thermal transition of ultraviolet absorbance at 260 mμ. Data on the ordinate are expressed in terms of molar absorbance based upon the concentration of the solution as determined from analysis of the ultraviolet spectrum after denaturation.[23] The molar absorbance of crater DNA is higher than that of control DNA before heating whereas the values are nearly equal after heating. Expression of the ordinate values in terms of relative absorbance, as is conventional for thermal transition curves, would lead to misinterpretation of these data. The arrows point to the Tm values. Note the small second transition Tm' at about 80°C exhibited by crater DNA.

DNA (Table V). The findings summarized above for the thermal transition curve at 260 mμ were confirmed. In addition, the mole fraction of A + T nucleotides was estimated from the parameter ϕ to be lower in the crater DNA: 53% vs 59% of the total A + T + G + C. The concentration of DNA estimated from the hyperchromic (difference) spectrum was less than that derived from the denatured spectrum for both DNA samples. The ratio of the concentrations from hyperchromic and denatured spectra may be considered an estimate of the fraction of the DNA sample which melts normally when heated; this fraction was smaller for crater DNA.

Examination of the absorption spectra obtained after heat denaturation showed for crater DNA a relative increase in absorbance at wavelengths both lower and higher than the major peak at 260 mμ. The absorbance ratios at 230 mμ/260 mμ and 290 mμ/260 mμ were higher in crater samples (Table V).

Intrinsic Viscosity

The intrinsic viscosities of crater and control DNA samples were equal when measured before or after heat denaturation (Table VI). However, the crater

Table V. Analysis of the ultraviolet absorption spectrum by the method of Hirschman and Felsenfeld.[23]

	Kangaroo rat		Long-Evans rat
	Crater	Control	
E_N (native)	6975	6758	6545
E_D (denatured)	9075	9112	9101
E_D/E_N	1.30	1.35	1.39
Fraction melting	0.733	0.835	0.938
Mole fraction $\frac{A+T}{A+T+C+G}$	0.532	0.593	0.624
A_{230}/A_{260}	0.492	0.441	0.423
A_{290}/A_{260}	0.222	0.200	0.194

Table VI. Intrinsic viscosity.

		Kangaroo rat liver		Long-Evans rat liver	Salmon sperm
		Crater	Control		
Native DNA	[η] dl/g	62	63	68	66
	Slope	0.61	0.26	1.98	0.96
Heat-denatured[a]	[η] dl/g	23.5	24.1	46.5	18.7
	Slope	0.030	0.075	0.19	0.28

[a]Concentrations of heat-denatured DNA were determined from A_{260} values measured before heating.

DNA before heating exhibited a substantially higher slope of the regression line relating reduced specific viscosity and DNA concentration.

Enhancement of Fluorescence of Ethidium Bromide

In the presence of DNA the fluorescence emission of ethidium bromide at 590 mμ is enhanced manyfold. The enhancement is approximately twice as great for native as for denatured DNA. No difference was observed between the enhancement by native crater and control DNA.

Primer-Template Activity of DNA

The primer-template activity of crater and control DNA samples was measured with four different polymerases (Table VII). In each instance the assays were conducted under conditions specified in the literature as optimal for the particular enzyme, and a saturating amount of DNA was added. For RNA polymerases from E. coli and M. lysodeikticus and DNA polymerase from regenerating rat liver, the template activity of crater DNA was consistently about 20% higher than control DNA. For DNA polymerase from E. coli there was no significant difference.

A single experiment was performed in which template activity was compared at a series of DNA concentrations (Fig. 5). Crater DNA was slightly lower in activity

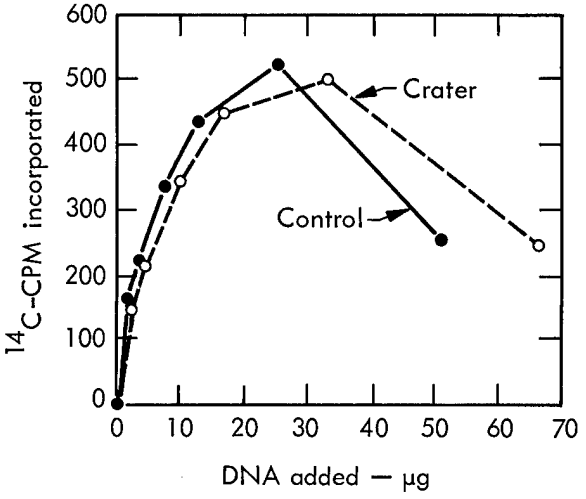

Fig. 5. DNA saturation curve for E. coli RNA polymerase.

Table VII. Assays of primer-template function.[a]

Enzyme	Source	DNA primer-template: Kangaroo rat Crater Net counts/min[b]	Kangaroo rat Control Net counts/min	Δ%	Long-Evans rat Net counts/min	Salmon sperm Net counts/min
RNA polymerase	E. coli	272	220	+23.6	199	173
RNA polymerase	M. lysodeikticus	845	726	+16.4	1273	423
DNA polymerase	E. coli	544	564	- 3.5	487	738
DNA polymerase[c]	Regenerating Long-Evans rat liver	718	592	+21.3	719	939

[a]The results were obtained by averaging the values from duplicate determinations on DNA samples from pooled livers of two sets of crater kangaroo rats and one set of control kangaroo rats. The values for DNA from Long-Evans rat and salmon sperm were from single determinations, but comparable to those obtained in many assays.

[b]Data are given in net counts/min of incorporated labeled precursor (ATP-^{14}C, dCTP-^{14}C or dTTP-^{14}C) after subtraction of the value for a vessel in which the enzyme was added terminally and followed immediately by TCA.

[c]The results with DNA polymerase from regenerating rat liver were from single experiments with DNA from crater and control kangaroo rats obtained a few months earlier but otherwise similar to those reported in full here.

than control DNA up to the maximal activity but higher than the control above the saturating amount of DNA. The reason for the decline in activity above the maximum is not known; it is only partly a result of self-absorption of ^{14}C counts by the larger amount of DNA-RNA precipitate on the filter.

Relative Incorporation of ATP-^{14}C and ATP-γ-^{32}P into RNA

Maitra et al.[32] have reported that RNA chains are initiated by RNA polymerase with the incorporation of a purine nucleotide triphosphate. The amount of ATP-γ-^{32}P incorporated should therefore be proportional to the number of RNA chains initiated. The amount of incorporation of ATP-^{14}C or other nucleotide labeled in the base moiety is proportional to the total amount of RNA synthesized. The ratio of $^{14}C/^{32}P$ incorporated is therefore considered to be an indication of the average length of RNA chains synthesized in the reaction.

The experiment reported in Fig. 6, with the exception of an aberrant point considered to represent technical error, indicated a lower $^{14}C/^{32}P$ incorporation ratio for crater DNA, which resulted from a relatively high ^{32}P incorporation. The ratio decreased slightly with increasing amount of added DNA, whereas the ratio for control DNA increased.

Susceptibility to Hydrolysis by DNase I

The rate of increase in ultraviolet absorbance at 260 mμ during incubation with DNase I was the same for crater and control DNA.

Discussion

PROPERTIES OF LIVER DNA FROM KANGAROO RATS

We have isolated DNA from Long-Evans rat and kangaroo rat livers by a method originally devised for calf thymus DNA. When allowance was made during the procedure for the much smaller DNA content of liver, a white fibrous product was obtained which had all of the expected physicochemical and primer-template properties of native DNA. The kangaroo rat liver DNA did not differ in any important respect from Long-Evans rat liver DNA or salmon sperm DNA.

NATURE AND DOSAGE OF RADIATION EXPOSURE OF CRATER KANGAROO RATS

The kangaroo rat is believed to live on a range only a few hundred feet in diameter, except during infrequent migration periods.[36] It consumes no free water, but derives nearly all of its water from its principal food, the seeds of the Russian thistle (Salsola kali). There may also be a small amount of respiratory water exchange via humid air in the burrows. The crater animals studied were trapped between 200 and 400 ft from the crater lip (800-1000 ft from ground

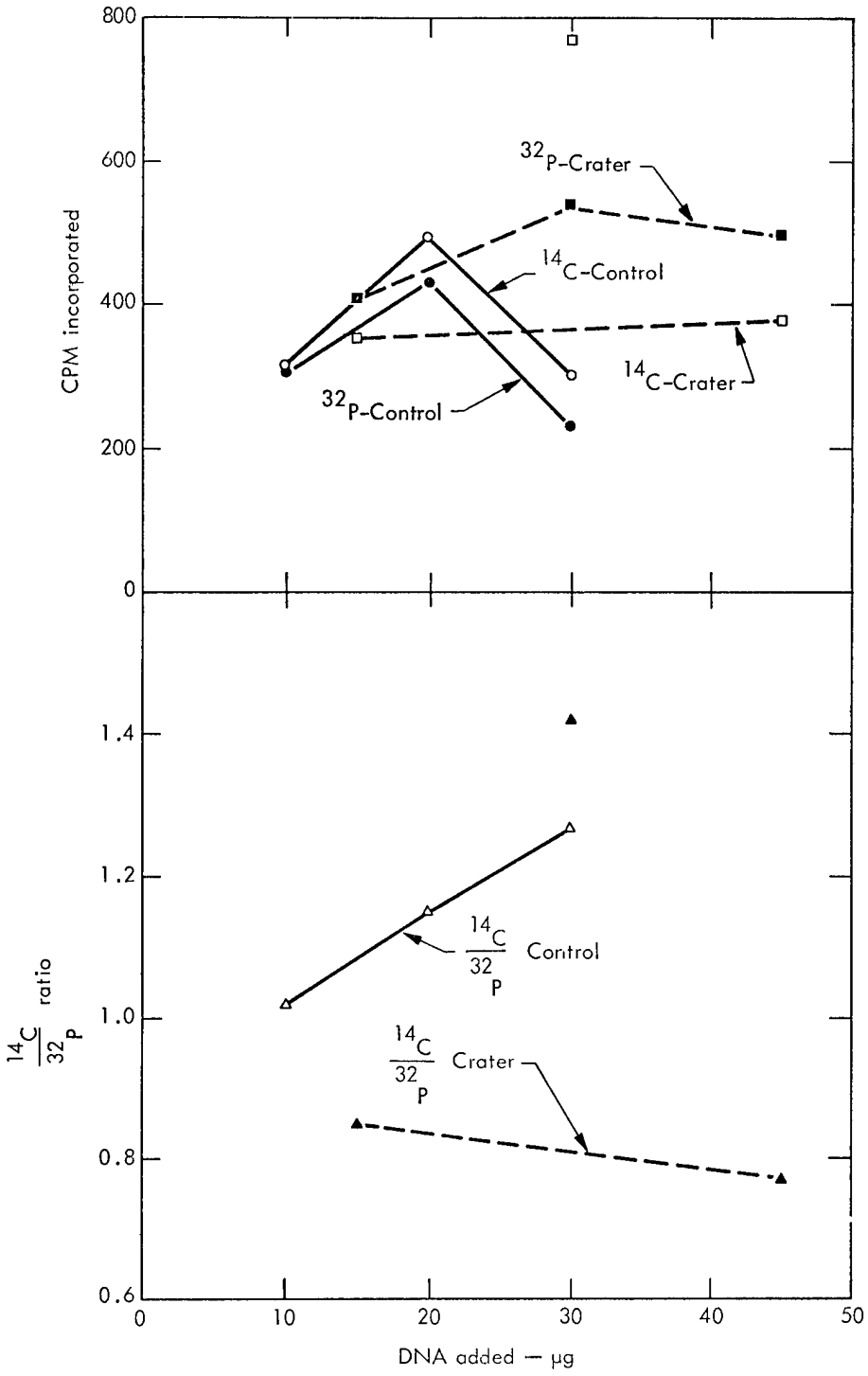

Fig. 6. DNA saturation curves for E. coli RNA polymerase with simultaneous incorporation of ATP-^{14}C (indicating total RNA synthesis) and ATP-γ-^{32}P (indicating RNA chains intiated). The very high incorporation of ^{14}C in the crater sample at 30 μg of DNA is believed to represent technical error. The results suggest that RNA polymerase may be bound to crater DNA at more sites with more RNA chains initiated but that the chains are of shorter average length.[32] The conclusion is tentative because of the limited amount of data and the apparent error in one vessel.

zero). Koranda has shown that the ^{3}H content of soil and plants remains markedly elevated to at least 2000 ft from ground zero.[11,12] The ^{3}H levels in the body water and tissues (including the liver DNA) of the animals studied correspond well to those found in the corresponding soil and plant samples. Therefore, it is probable that most of our animals have lived their entire lives from the time of conception in this uniformly tritiated environment. Thus all cellular constituents, regardless of their metabolic turnover rates, should contain ^{3}H in proportion to their hydrogen content. These circumstances are unique and difficult to duplicate under laboratory conditions in a mammal.

With regard to possible radiation effects on DNA, a special question arises whether the irradiation from decay of ^{3}H atoms within the DNA molecule might have any different effect from that from ^{3}H in the surrounding water and proteins. We do not know the answer, but have assumed no special properties in our dose calculations. Likewise we have not assumed that the R. B. E. of ^{3}H irradiation differs from 1.0.[37]

Most of the other radionuclides produced in the explosion had decayed by the time the animals were trapped. The total dosage from external β and γ radiation was estimated at 26 rad per year, approximately equal to that from ^{3}H. The amounts of γ-emitting radionuclides (principally ^{90}Sr and ^{137}Cs) incorporated in the plant and animal tissues do not differ appreciably from those prevalent elsewhere in the U. S.

COMPARISON OF LIVER DNA FROM CRATER AND CONTROL ANIMALS

Although the DNA samples after isolation had the same gross appearance and approximately the same yield per gram of liver, it was noted repeatedly during the purification procedure that the dissociated protein at the two precipitation steps was more voluminous and more granular in appearance in the crater samples. At present we have no further information about this observation.

The differences and similarities between crater and control DNA samples are summarized in Table VIII. Many or all of the observed differences may be interrelated, but no definitive interpretation in terms of structural alteration in the DNA is possible yet.

The elevated protein and histone content of crater DNA might result from covalent crosslinking to DNA or some form of strong noncovalent binding. This might also account for the secondary thermal transition (Tm') above the main transition (Tm) as described by Von Hippel et al. with model complexes of DNA and polyamino acids or protamine.[38,39] Alternately the Tm' could be accounted for by the presence of a subfraction of DNA with altered properties[40,41] or by the presence of a small number of altered base pairs with increased binding energy.

The elevated molar absorbance of unheated crater DNA and its narrower and smaller thermal transition suggest that the crater DNA as isolated may be already slightly unfolded or "denatured." However, apparently not enough single-strand breaks were induced by the radiation to lower the viscosity significantly

Table VIII. Summary of observations.

Crater DNA contrasted with control DNA
1. ^{3}H content of 6×10^{6} dis/min/g H.
2. Chronic radiation exposure averaging 10 rad from internal ^{3}H and 10 rad from external β and γ radiation.
3. Higher protein and histone content.
4. Higher molar absorbance in native state.
5. Lower thermal transition magnitude.
6. Narrower thermal transition.
7. Secondary thermal transition (Tm').
8. Lower mole fraction of A + T nucleotides.
9. Increased absorbance ratios at 230 mμ/260 mμ and 290 mμ/260 mμ.
10. Increased concentration-dependence of reduced specific viscosity.
11. Increased template activity for 3 polymerases.
12. Decreased incorporation ratio of ATP-^{14}C/ATP-γ-^{32}P.

No difference observed
1. Wavelength of maximum absorbance.
2. Molar absorbance after heat denaturation.
3. Thermal transition midpoint (Tm).
4. Intrinsic viscosity before or after heating.
5. Enhancement of fluorescence of ethidium bromide.
6. Template activity for E. coli DNA polymerase.
7. Susceptibility to hydrolysis by DNase I.

after heat denaturation, as was observed by Harrington with 1000 r of x-irradiation.[43] The increased concentration-dependence of the viscosity of unheated crater DNA is also consistent with some type of distortion of the molecule without actual fragmentation, since concentration-dependence probably reflects intermolecular interactions.

The lower mole fraction (ϕ) of A + T nucleotides and the higher absorbance ratios at 230 mμ/260 mμ and 290 mμ/260 mμ for crater DNA can be calculated from the absorption spectra taken either before or after heating. A simple explanation might be the spectral contribution of the extra protein moiety found in crater DNA; however an approximate calculation shows that much more protein than was found would be required to account for the data. Selective destruction or transformation of nucleotides could produce the changes. The approximately equal molar absorbances at 260 mμ of crater and control DNA observed after heat denaturation excludes anything more than a slight destruction of the total ultraviolet-absorbing material. Our preliminary search for transformed nucleotides by thin-layer chromatography

has not revealed any, but we have not yet achieved optimal sensitivity of the method. The possible destruction or transformation of nucleotides by irradiation has important implications for genetic or somatic mutations. Therefore, these observations will be further investigated.

Preliminary experiments in our laboratory have shown changes in ϕ and in the absorbance ratios similar to those described above when purified DNA was irradiated in vitro with 1000 r of γ-radiation from ^{137}Cs, and when poly-dAT which had been synthesized from ^{3}H-TTP of high specific activity was subjected to self-irradiation.

The enhanced primer-template activity of crater DNA for three polymerases when saturating amounts of DNA were added is difficult to understand. Unfortunately to date we have only one satisfactory experiment covering a wide range of DNA concentrations; this experiment indicates that the enhancement may apply only to high DNA levels.

Harrington has carried out the most extensive studies of the effects of ionizing radiation on the template activity of DNA.[42-44] Most of her experiments were carried out with an assay system consisting of purified DNA from calf thymus and DNA polymerase from E. coli. The radiation dose, administered in 0.01 M phosphate buffer, was usually 1000 r of x rays, although a small effect was observed at 100 r. The radiation effect was complex. There was slight enhancement of template activity over unirradiated DNA at low concentrations of DNA and impairment at higher concentrations. The maximal activity for irradiated DNA was less than that for unirradiated DNA and was observed at a lower DNA concentration.

Physicochemical studies by Harrington showed that specific viscosity was moderately decreased in unheated, irradiated (1000 r) DNA and markedly decreased in heated, irradiated DNA. There was also a moderate decrease in ultracentrifugal sedimentation velocity and a 1.5°C decrease in the thermal transition midpoint (Tm). The irradiated DNA was more susceptible to hydrolysis by a nuclease preparation from E. coli. The findings were interpreted as showing the production of a small number of single- and double-strand breaks in the DNA molecules by the radiation. Similar but less striking results were produced by in vivo irradiation (1000 r) of leukemic lymphoblast cell cultures.

Treatment of DNA with deoxyribonucleases I and II and ultrasound increased template activity at all DNA concentrations; ultraviolet light decreased template activity at all DNA concentrations. The effect of heat denaturation more closely resembled that of x rays in that there was little difference in template activity at low DNA concentrations and a decrease at higher DNA concentrations.

Stacey showed inhibition of template activity for calf thymus DNA polymerase of calf thymus DNA that had received 20,000 or more rad of x-radiation.[45] Both control and irradiated samples required heat denaturation before becoming active for this enzyme.

Zimmermann et al.[46] and Kroger et al.[47] showed that x-ray doses of 1000 rad or more inhibited the template activity of DNA for RNA polymerase prepared from E. coli. On the other hand the synthesis of

polyadenylic acid or polyuridylic acid by RNA polymerase was increased after γ-irradiation at kilorad doses to the DNA template. Homopolymer synthesis is normally much higher with heat-denatured than with native DNA.

Weiss and Wheeler demonstrated approximately 50% inhibition of template activity for M. lysodeikticus RNA polymerase of calf thymus DNA that had received 10,000 rad of γ-irradiation.[48] The base ratios in the synthesized RNA were altered with the irradiated template so that less CMP and more AMP and GMP were incorporated. The results were interpreted in terms of production by the radiation of sites on the DNA which could bind RNA polymerase but at which little or no RNA was synthesized. The altered RNA base ratios were thought to represent coding errors resulting from damage to bases in the DNA.

When ATP-γ-^{32}P and ATP-^{14}C were simultaneously added to the incubation mixtures, the ratio of ^{14}C/^{32}P incorporation was lower for crater DNA owing to a relatively high ^{32}P incorporation. Our present interpretation of these data is that the crater DNA possessed an increased number of binding sites for the polymerase where RNA chains were initiated with the terminal nucleotide triphosphate. Although total RNA synthesis was about equal on both DNA samples (when a saturating amount of DNA was added), the average amount of synthesis (RNA chain length) at each site was decreased on the crater DNA template. According to the hypothesis proposed by Weiss and Wheeler,[48] additional sites may be produced in DNA by irradiation, at which RNA polymerase molecules are bound, with incorporation of intact ATP at the start of RNA chains but with little or no synthesis of RNA (incorporation of AMP). Whether the additional chain-initiation sites observed in our experiments resulted from single-strand breaks in the DNA chain or from other local structural changes is unknown.

In summary, we have demonstrated that DNA isolated from the livers of kangaroo rats exposed to continuous low-dose irradiation exhibits (1) minor structural alterations, which are tentatively interpreted as indicating partial unfolding with exposure of additional binding sites for RNA polymerase, and (2) altered performance as a template for certain DNA and RNA polymerases.

POSSIBLE CAUSES FOR THE DIFFERENCES BETWEEN CRATER AND CONTROL DNA

Ionizing Radiation

We believe that the β-radiation from ^{3}H is the most likely cause of the alterations observed in crater DNA samples. Some additional contribution from the external γ-radiation cannot be excluded. The effects probably are derived from ^{3}H atoms both within the DNA molecules and in the surrounding water and protein. There appear to be three possible modes of action of the radiation: (1) direct effects upon DNA structure resulting possibly in a small number of single-strand breaks and in localized disruptions of base-stacking, (2) indirect effects in which unspecified radiation effects lead to a differential fragility of the DNA samples during the isolation procedure, so that DNA isolated from the crater animals is a biased sample of liver cell DNA, or (3) indirect effects

owing to the activation of in vivo repair mechanisms which lead to the observed differences in DNA properties.

To test the third hypothesis preliminary experiments, not reported here in detail, showed that neither the incorporation of inorganic $^{32}PO_4$ in vivo nor of dATP in an in vitro assay for liver DNA polymerase activity was enhanced in crater animals. The calculations presented in Table II, showing that there were on the average only 1.5 ^{3}H-disintegrations in the DNA per cell per year, show that one should not expect to be able to measure directly such "repair" activities in any short-term assay.

Geographic or Nutritional Differences

The crater and control animals were trapped in similar desert environments approximately 40 miles apart. By simple taxonomic criteria[49] the species appeared to be identical. The food supply around Sedan crater consisted almost exclusively of Salsola kali, which is the preferred food of these animals. Additional food plants were available at the control sites. It is conceivable, though unlikely, that a difference in nutrient intake was responsible for our findings on liver DNA.

Technical Artifacts

The properties of DNA isolated from several pools of crater or control animals over a period of nearly two years were not entirely reproducible. Unfortunately the observed differences between crater and control DNA were small and not much greater than the internal variation in properties. However, in each simultaneously prepared batch of crater and control DNA the differences observed were always in the same direction for each variable examined. Although the isolation of DNA is a lengthy and complex procedure, we believe it is unlikely that artifacts of technique could be responsible for the differences consistently observed.

Conclusions

There is an abundant population of kangaroo rats living on the throwout zone around Sedan crater. They appear to grow and reproduce normally, and they survive well in captivity. They are usually trapped in greater yield than the control animals, which may indicate a relative protection from natural enemies that is perhaps due to the limited variety of foods in the crater area. Under present circumstances, more detailed knowledge of their life cycle, longevity, and incidence of mutations and disease is not available.

The animals from the crater environment were exposed to approximately equal amounts of internal soft β and external γ irradiations which provided a chronic dosage totaling 20-50 rad for adult animals. This level appraches that which could be encountered in peaceful applications of nuclear energy. At the level observed in this study one might expect to see a slight increase in mutation rate and chromosome aberrations, based on previous studies. However, such doses, whether acute or chronic, have not previously to our

knowledge produced effects which could be demonstrated in vitro in reasonably well understood biochemical systems. Previous biochemical studies have generally required acute radiation doses at least one or two orders of magnitude higher to produce observable effects.

The physicochemical and functional changes observed in the DNA of the irradiated animals were small but they appeared to be abnormal by comparison with control samples and samples from Long-Evans rats and other organisms. We cannot conclude that these changes were harmful, because the population in which they were observed was apparently a vigorous population. However, we must conclude that such changes are potentially harmful since they indicate an altered conformation of the DNA, which, in the mammalian liver cell, regulates metabolism by messenger transcription and could give rise to somatic mutations leading to cell death or neoplastic transformation.

Further study must be directed particularly at the template performance of this irradiated DNA with respect to transcription errors and response to stimuli for DNA replication. The logistic difficulties and the inability to vary radiation type and dosage in the present studies suggest that a shift to a laboratory animal will be advisable.

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References

1. M. A. Bender, P. C. Gooch, and D. M. Prescott, "Aberrations Induced in Human Leukocyte Chromosomes by ^{3}H-Labeled Nucleosides," Cytogenetics 1, 65-74 (1962).
2. M. Yamada and T. T. Puck, "Action of Radiation on Mammalian Cells. IV. Reversible Mitotic Lag in the S3 Hela Cell Produced by Low Doses of X-rays," Proc. Natl. Acad. Sci. (U.S.) 47, 1181-1191 (1961).
3. T. T. Puck, "Cell Turnover in Mammalian Tissues: Use of Cell Depletion Measurements to Calculate X-ray Reproductive Survival Curves in vivo," Proc. Natl. Acad. Sci. (U.S.) 54, 1797-1803 (1965).
4. R. J. Berry, R. Oliver, and A. B. Reiskin, "Reproductive Death of Mammalian Cells Due to Beta-Radiation from Incorporated Thymidine Labeled with ^{3}H or ^{14}C," Health Phys. 12, 1461-1466 (1966).
5. F. Hutchinson, "The Molecular Basis for Radiation Effects on Cells," Cancer Res. 26, 2045-2052 (1966).
6. R. H. Haynes, "Molecular Localization of Radiation Damage Relevant to Bacterial Inactivation," in International Symposium on Physical Processes in Radiation Biology, L. Augenstein, R. Nason and B. Rosenberg, Eds. (Academic Press, New York, N.Y., 1964), pp. 51-68.
7. W. Troll, S. Belman, E. Berkowitz, Z. F. Chmielewicz, J. L. Ambrus, and T. J. Bardos, "Differential Responses of DNA and RNA Polymerase to Modifications of the Template Rat Liver DNA Caused by Action of the Carcinogen Acetylaminofluorene in vivo and in vitro," Biochim. Biophys. Acta 157, 16-24 (1968).
8. S. Omata and S. Ichii, "Priming Activity for RNA Synthesis of Chromatin from Transplantable Hepatomas and Livers from Tumor-Bearing Rats," Gann 58, 55-59 (1967).
9. T. J. Bardos, J. L. Ambrus, Z. F. Chmielewicz, A. G. Penny, and C. M. Ambrus, "Biochemical Parameters of Neoplasia. I. 'Priming' Activity of Deoxyribonucleic Acid from Normal and Neoplastic Human Tissues in a Deoxyribonucleic Acid Polymerase System," Cancer Res. 25, 1238-1243 (1965).
10. R. J. Fiel, T. J. Bardos, Z. F. Chmielewicz, and J. L. Ambrus, "Biochemical Parameters of Neoplasia. II. Some Macromolecular Properties of Deoxyribonucleic Acid from Normal and Neoplastic Tissue," Cancer Res. 25, 1244-1253 (1965).
11. J. J. Koranda, Residual Tritium at Sedan Crater, Lawrence Radiation Laboratory, Livermore, Rept. UCRL-70292 (1967).
12. J. J. Koranda, J. R. Martin, and R. Wikkerink, Residual Tritium at Sedan Crater. II. Soil and Ejecta Studies, Lawrence Radiation Laboratory, Livermore, Rept. UCRL-50360 (1967).

13. C. D. Jorgensen, D. M. Allred, and D. E. Beck, "Some Effects of an Underground Nuclear Detonation on Biotic Communities at the Nevada Test Site," Proc. Utah Acad. Sci., Arts, Letters 40, Pt. I, 49-61 (1963).
14. J. J. Koranda, J. R. Martin, and R. Wikkerink, Biological Half-Life Studies of Tritium in Animals with a Unique Chronic Exposure at Sedan Crater, Lawrence Radiation Laboratory, Livermore, Rept. UCRL-70914 Abstract (1968).
15. E. R. M. Kay, N. S. Simmons, and A. L. Dounce, "An Improved Preparation of Sodium Desoxyribonucleate," J. Am. Chem. Soc. 74, 1724-1726 (1952).
16. J. Marmur, "A Procedure for the Isolation of Deoxyribonucleic Acid from Microorganisms," J. Mol. Biol. 3, 208-218 (1961).
17. R. Mantsavinos, "Studies on the Synthesis of Deoxyribonucleic Acid by Mammalian Enzymes. I. Incorporation of Deoxyribonucleoside 5'-Triphosphates into Deoxyribonucleic Acid by a Partially Purified Enzyme from Regenerating Rat Liver," J. Biol. Chem. 239, 3431-3435 (1964).
18. O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall, "Protein Measurement with the Folin Phenol Reagent," J. Biol. Chem. 193, 265-275 (1951).
19. L. D. G. Hamilton, in A Laboratory Manual of Analytical Methods of Protein Chemistry, Vol. 2, P. Alexander and R. J. Block, Eds. (Pergamon Press, New York, 1960), p. 95.
20. W. E. Cohn, E. Volkin, and J. X. Khym, "Separation of 5'-Deoxyribonucleotides," Biochem. Preps. 5, 49-55 (1957).
21. K. Randerath and E. Randerath, "Ion-Exchange Thin-Layer Chromatography. XV. Preparation, Properties and Applications of Paper-Like PEI-Cellulose Sheets," J. Chromatog. 22, 110-117 (1966).
22. G. R. Bartlett, "Phosphorus Assay in Column Chromatography," J. Biol. Chem. 234, 466-486 (1959).
23. S. Z. Hirschman and G. Felsenfeld, "Determination of DNA Composition and Concentration by Spectral Analysis," J. Mol. Biol. 16, 347-358 (1966).
24. B. H. Zimm and D. M. Crothers, "Simplified Rotating Cylinder Viscometer for DNA," Proc. Natl. Acad. Sci. (U.S.) 48, 905-911 (1962).
25. D. M. Crothers and B. H. Zimm, "Viscosity and Sedimentation of the DNA from Bacteriophages T2 and T7 and the Relation to Molecular Weight," J. Mol. Biol. 12, 525-536 (1965).
26. G. A. Bray, "A Simple Efficient Liquid Scintillator for Counting Aqueous Solutions in a Liquid Scintillation Counter," Anal. Biochem. 1, 279-285 (1960).
27. J. B. LePecq and C. Paoletti, "A New Fluorometric Method for RNA and DNA Determination," Anal. Biochem. 17, 100-107 (1966).
28. J. B. LePecq and C. Paoletti, "A Fluorescent Complex Between Ethidium Bromide and Nucleic Acids," J. Mol. Biol. 27, 87-106 (1967).
29. M. Chamberlin and P. Berg, "Deoxyribonucleic Acid-Directed Synthesis of Ribonucleic Acid by an Enzyme from Escherichia coli," Proc. Natl. Acad. Sci. (U.S.) 48, 81-94 (1962).

30. T. Nakamoto, C. F. Fox, and S. B. Weiss, "Enzymatic Synthesis of Ribonucleic Acid. I. Preparation of Ribonucleic Acid Polymerase from Extracts of Micrococcus lysodeikticus," J. Biol. Chem. 239, 167-174 (1964).
31. C. C. Richardson, C. L. Schildkraut, H. V. Aposhian, and A. Kornberg, "Enzymatic Synthesis of Deoxyribonucleic Acid. XIV. Further Purification and Properties of Deoxyribonucleic Acid Polymerase of Escherichia coli," J. Biol. Chem. 239, 222-232 (1964).
32. U. Maitra, Y. Nakata, and J. Hurwitz, "The Role of Deoxyribonucleic Acid in Ribonucleic Acid Synthesis. XIV. A Study of the Initiation of Ribonucleic Acid Synthesis," J. Biol. Chem. 242, 4908-4918 (1967).
33. M. Kunitz, "Crystalline Deoxyribonuclease. I. Isolation and General Properties, Spectrophotometric Method for the Measurement of Desoxyribonuclease Activity," J. Gen. Physiol. 33, 349-362 (1950).
34. N. R. French, B. G. Maza, and A. P. Aschwanden, "Life Spans of Dipodomys and Perognathus in the Mojave Desert," J. Mammology 48, 537-548 (1967).
35. E. P. Geiduschek, "On the Factors Controlling the Reversibility of DNA Denaturation," J. Mol. Biol. 4, 467-487 (1962).
36. D. M. Allred and D. E. Beck, "Range of Movement and Dispersal of some Rodents at the Nevada Atomic Test Site," J. Mammalogy 44, 190-200 (1963).
37. V. P. Bond and L. E. Feinendegen, "Intranuclear ^{3}H Thymidine: Dosimetric, Radiobiological and Radiation Protection Aspects," Health Phys. 12, 1007-1020 (1966).
38. D. E. Olins, A. L. Olins, and P. H. Von Hippel, "Model Nucleoprotein Complexes: Studies on the Interaction of Cationic Homopolypeptides with DNA," J. Mol. Biol. 24, 151-176 (1967).
39. D. E. Olins, A. L. Olins, and P. H. Von Hippel, "On the Structure and Stability of DNA-Protamine and DNA-Polypeptide Complexes," J. Mol. Biol. 33, 265-281 (1968).
40. V. I. Tokarskaya and S. R. Umanskii, "Physicochemical Properties of DNA Fractions," Molecular Biology (U.S.S.R.) 1, 433-438 (1967)(English translation).
41. F. Quetier and E. Guille, "Studies on Plant Deoxyribonucleic Acids. I. Extraction, Fractionation and Analytical Thermal Denaturation," Arch. Biochem. Biophys. 124, 1-11 (1968).
42. H. Harrington, "Effect of X-Irradiation on the Priming Activity of DNA," Proc. Natl. Acad. Sci. (U.S.) 51, 59-66 (1964).
43. H. Harrington, "Effect of X-Irradiation on the Physicochemical Properties of Deoxyribonucleic Acid," Arch. Biochem. Biophys. 117, 615-622 (1966).
44. H. Harrington, "Effect of Various Agents on the Priming Activity of DNA for Escherichia coli DNA Polymerase," J. Mol. Biol. 15, 152-159 (1966).
45. K. A. Stacey, "The Inhibition of DNA Synthesis by Calf-Thymus Polymerase by X-Irradiation of the Primer DNA," Biochem. Biophys. Res. Commun. 5, 486-490 (1961).

46. F. Zimmermann, H. Kröger, U. Hagen, and K. Keck, "The Effect of X-Irradiation on the Priming Ability of DNA in the RNA Polymerase System," Biochim. Biophys. Acta 87, 160-162 (1964).

47. H. Kröger, L. Schuchmann, E. Petersen, and U. Hagen, "Studies on Homopolymer Synthesis with Irradiated DNA in the RNA Polymerase System," Biochim. Biophys. Acta 142, 542-544 (1967).

48. J. J. Weiss and C. M. Wheeler, "Some Studies on γ-Irradiated DNA as Primer in RNA Synthesis," Biochim. Biophys. Acta 145, 68-75 (1967).

49. E. R. Hall and K. R. Kelson, Mammals of North America, Vol. I (Ronald Press, New York, 1959), p. 530.

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